

ADVANCED USE OF FTIR SPECTRA OF MILK FOR FEEDING AND HEALTH MANAGEMENT

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INTRODUCTION

Classically, information from the mid-infrared (mid-IR) spectra is used to measure fat, protein, and lactose content of milk for payment and DHIA testing. The first generation of instruments used optical filters to select for specific wavelengths of infrared light absorbed by key chemical bonds in fat, protein, and lactose in milk. In the mid to late 1990's, the first commercial Fourier Transform Infrared (FTIR) milk analyzers were marketed. FTIR instruments used a different approach to collect information by producing a full infrared spectra of each sample. The information at the classical wavelengths for measurement of fat, protein and lactose could be extracted from that spectra and allowed the speed (samples per hour) of analysis to be increased.

There are many secrets hidden within the mid-IR spectra and only recently have we started to understand how to reveal them using advanced statistical methods. The first secret is the wealth of information about milk fatty acid composition that could be useful for nutrition and feeding management. We have just scratched the surface of other predictive information that can be extracted from the spectra that may allow a more proactive management of individual cow health and reproduction. Today, I will focus on the use of milk fatty acid (FA) information for feeding management of dairy cows and report the status of a major on-going research project. Our objectives were: 1) measure FA composition of individual producer milks using new chemometric models for FTIR milk analysis and 2) determine if there are correlations between milk FA composition and bulk tank milk fat and protein tests.

EXPERIMENTAL APPROACH

Prior to the current study a group of partial least squares (PLS) chemometric prediction models were developed from mid-IR spectra. The spectra of modified milk calibration samples (Kalyejian et al., 2006a,b), bulk tank milks, and individual cow milks were used in combination with chemical reference chemistry for fat (AOAC, 2000; method 989.05; 33.2.26), total protein (AOAC, 2000; method 991.20; 33.2.11 and nonprotein nitrogen (AOAC, 2000; method 991.21; 33.2.12) with true protein calculated by difference, anhydrous lactose (Lynch et al., 2007) and gas liquid chromatography (Barbano and Sherbon, 1980; Lynch et al., 1992) for FA analysis using a Varian CP-SIL88 capillary column [(100m x 0.25 mm x 0.2 µm film thickness), ID code # CP7489; Varian, Inc., Lake Forest, CA], installed in a Hewlett Packard 6890 GC System equipped with an automatic liquid sampler and a flame ionization detector (Hewlett Packard Co., Wilmington, DE). A library of chemometric prediction models for the major

components in milk and milk FA composition for use on a Lactoscope FTA (Delta Instruments, Drachten, The Netherlands) has been developed. A variety of individual FA and groups of FA were measured. The following individual FA were measured by mid-IR: C16:0; C18:0; C18:1 *cis*9, *cis*12; C18:1 *trans* 10; and C18:1 *trans* 11. The following groups of FA were measured: total FA; DeNovo (C4:0 to C14:0), mixed origin (C16:0, C16:1, C17:0), preformed (C18:0 and longer); total unsaturated FA, total *cis* FA; total *trans* FA; mono unsaturated FA; and poly unsaturated FA. All FA measures produce results from the IR in grams of FA per 100 grams of milk. Some researchers have used the grouping of FA as short, medium, and long chain FA but the exact definition of those groups varies among researchers. The group definitions of *DeNovo*, mixed origin, and preformed FA is much more clear and consistent because they are based on the biochemical pathways for FA synthesis and have better potential to be correlated with the biology, metabolism, and feeding of dairy cows.

In addition to the measures of FA concentrations, two fat concentration independent measures of FA structure were also done on each sample: mean FA chain length (expressed as mean carbon number per FA) and mean FA unsaturation (expressed as double bonds per FA). The measure of total FA (not fat) in g/ 100 g of milk is used as a new basis for a more accurate measurement of total fat content in the milk. This approach eliminates most of the weakness of traditional measures of fat by IR using the Fat A (C=O stretch) and Fat B (C-H stretch) because it compensates sample by sample for differences in FA composition when trying to estimate the total fat content of the milk in comparison to ether extraction (Kaylegian et al., 2009a,b). The relative proportion of the total FA in milk that are represented by an individual or group of FA can be expressed on a relative basis as a percent of total FA in the sample. Thus, it is possible to produce a simulated gas chromatograph FA analysis of milk fat directly from the same (IR spectra) of milk tested on the IR for fat, protein, and lactose concentration. Validation of IR FA results was done split sample analysis to compare IR and GLC FA estimates on samples through out the study. The manual model Delta Instruments Lactoscope FTA used in this study provided measurements of milk components and FA composition at a rate of about 100 milks per hour. A larger automated FTIR instrument can operate at about 6 times this speed. Reference testing for FA composition by GLC takes approximately 3 days to analyze 18 milks.

The calibration adjustment of the fat, true protein, anhydrous lactose and all FA measures on the IR milk analyzer is done once per month using a set of 14 modified milks described by Kaylegian et al. (2006a,b) that has reference values in (g FA per 100 g of milk) for each of the individual or groups of FA measured. The set of calibration samples is produced monthly at Cornell and was used to check the calibrations during the month.

Bulk tank milks from 430 farms located in Northern Vermont and Northeastern New York State were sampled and tested 3 to 20 times per month per farm for 15 months during the period from June 2012 to August 2013 using mid-FTIR (Lactoscope FTA, Delta Instruments, The Netherlands) for fat, protein, lactose and FA composition. FA data were organized and analyzed by breed: Jersey and Holstein.

RESULTS

The data from this survey of milk FA composition represents a wide range of farm managements practices and a wide range of herd sizes (herds that deliver about 10,000 pounds of milk per month to herds that deliver over 3 million pounds of milk per month). The present study is an observational survey that was intended to provide a view of milk FA composition of milk fat and and detect correlations between milk fat and protein concentration in bulk tank milk and milk FA composition on a large population of farms over a period of at least one year. The study produced a large amount of data and it cannot all be shown here. Therefore, the most interesting observations have been selected for presentation. In general, FA composition within farm from day-to-day was fairly consistent. When there was a major change in FA composition within a farm, it was usually due to a major change in feeding that shifted the FA composition. There was an overall breed (Holstein vs. Jersey) difference in FA composition, but there was a large amount of variation within each breed. Overall, the level of trans FA was not high in this population, particularly the C18:1 *trans* 10. Indicating that for the most part, classical milk fat depression was not a common problem in this population of farms, but there were some exceptions. The most interesting parameters in the FA data that were correlated with the concentration of fat and true protein in the bulk milk were the groups of FA (*DeNovo*, mixed origin, and preformed FA) and that is the primary focus of the results presented in this paper.

Milk Fat

The relationship between g/100 g of milk of *DeNovo* FA and bulk tank milk fat content is shown (Figure 1). There is a positive correlation of increasing bulk tank fat test with increasing *DeNovo* FA concentration in milk for both Holstein and Jersey milks. These FA are synthesized from the betahydroxy butyrate, acetate, and propionate produced in the rumen by forage fermentation and are transported via the blood, taken up by the mammary cells and used to synthesize the *DeNovo* FA and about half of the C16:0. The linear regression equation is located in the lower left hand corner of the graphs and the slope is about 2.2 to 2.3.

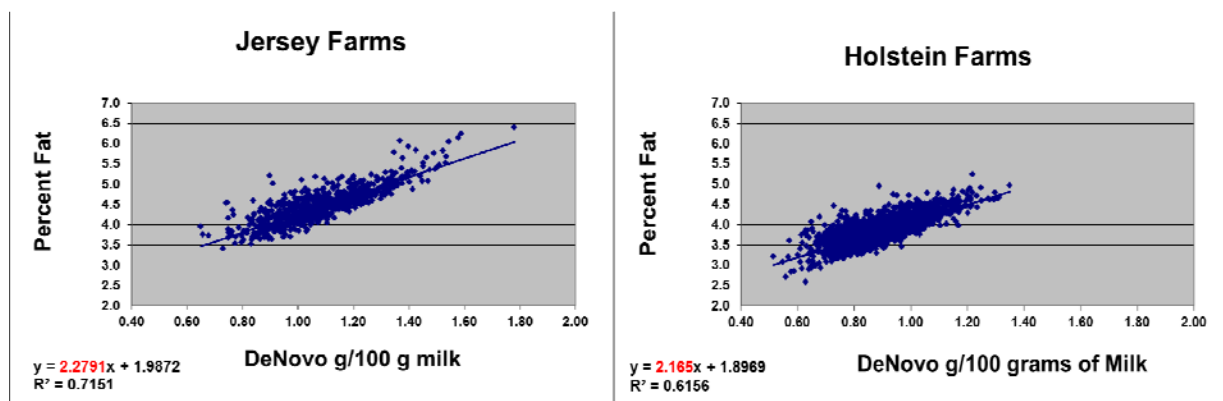


Figure 1. Bulk tank fat test (%) for Jersey and Holstein herds plotted as a function of milk *DeNovo* fatty acid content.

The relationship between the g/100 g of milk of mixed origin FA and bulk tank fat milk fat content is shown in Figure 2. There is a positive correlation of increasing bulk tank fat test with increasing mixed origin FA concentration in milk in both Holstein and Jersey milks. Palmitic acid (C16:0) is the major FA in this group. It can come preformed from the diet, or it can be synthesized in mammary cells *DeNovo* from acetate. The relative contribution of these two origins of C16:0 changes systematically with stage of lactation. In early lactation the C16:0 is primarily from mobilized body fat (i.e., preformed), but after a cow achieves positive energy balance, more of the C16:0 in the milk should be produced within the mammary cells from acetate (i.e., *DeNovo*). The linear regression is located in the lower left hand corner of the graphs and the slope is about 1.8 to 1.9, which is lower than the slope for the *DeNovo* FA.

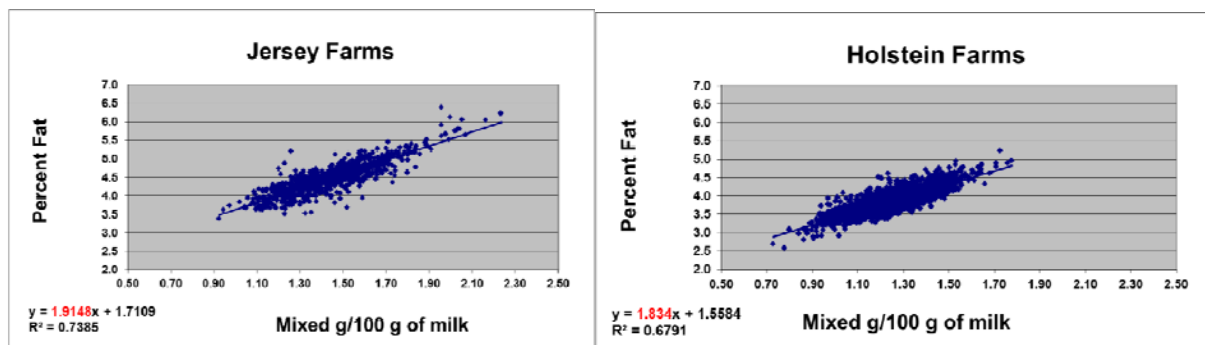


Figure 2. Bulk tank fat test (%) for Jersey and Holstein herds plotted as a function of milk mixed origin fatty acid content.

The relationship between the g/100 g of milk of performed FA and bulk tank fat milk fat content is shown in Figure 3. There is a positive correlation of increasing bulk tank fat test with increasing mixed origin FA concentration in milk for both Holstein and Jersey milks. The linear regression is located in the lower left had corner of the graphs and the slope is about 1.2 to 1.3, which is much lower than the slope for the *DeNovo* and mixed origin FA.

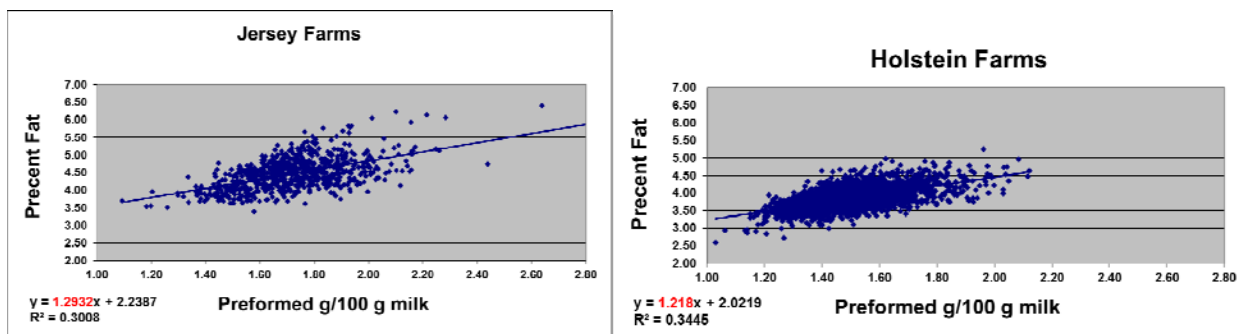


Figure 3. Bulk tank fat test (%) for Jersey and Holstein herds plotted as a function of milk preformed fatty acid content.

The much lower slope for the preformed (Figure 3) than then *DeNovo* FA (Figure 1) would seem to indicate that increasing the concentration of *DeNovo* FA in milk will produce a more rapid increase in total fat in the milk than inceasing preformed FA

(dietary origin). Enhancing production of *DeNovo* FA and increasing fat test in the bulk tank should be related to forage quality and efficiency of forage fermentation and digestion in the rumen.

Another independent measure of the characteristics of milk fat is the measurement of the average chain length and degree of unsaturation of the milk FA. These measures of fat structure by FTIR are independent of fat concentration in the milk. The relationships between mean FA carbon number and double bonds per fatty FA and bulk tank fat milk fat content are shown in Figures 4 and 5.

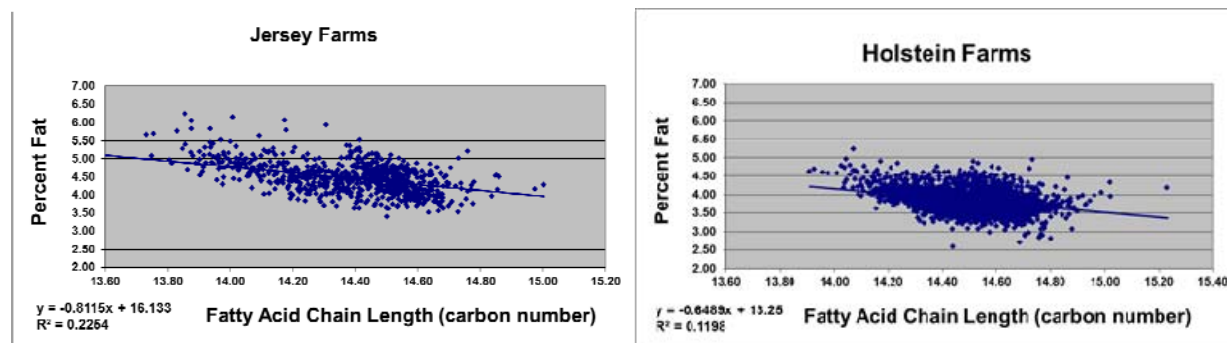


Figure 4. Bulk tank fat test (%) for Jersey and Holstein herds plotted as a function of milk FA carbon number per FA.

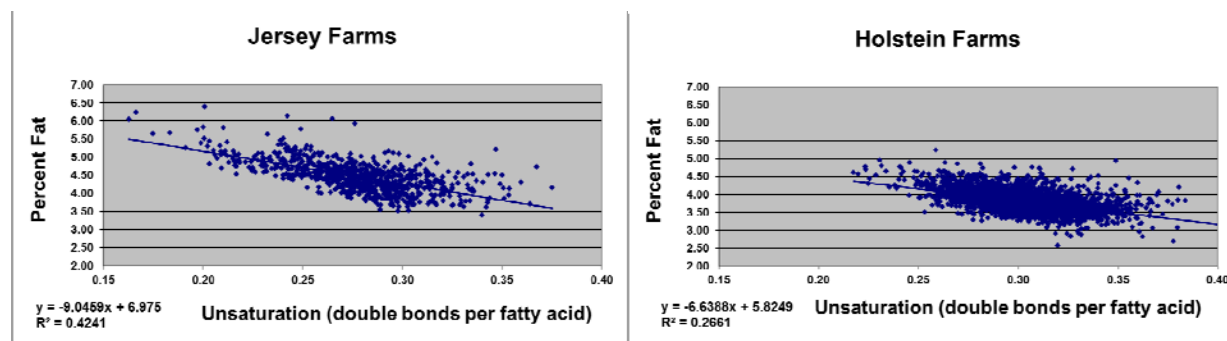


Figure 5. Bulk tank fat test (%) for Jersey and Holstein herds plotted as a function of milk fatty acid double bonds per fatty acid.

As the proportion of preformed FA in milk fat increases, it would be expected that the average FA chain length would increase. Thus, from the relationships shown in Figures 1, 2, and 3, it would be expected that milk fat concentration in bulk milk would decrease as FA chain length increased and this is what is shown in Figure 4. In general in milk fat, increased FA chain length is positively correlated with increased unsaturation (i.e., double bonds per FA). Therefore, as the observed amount of unsaturation in the milk increased in the population of 430 farms, the percent fat in the bulk tank decreased (Figure 5). The higher level of preformed FA in the milk fat could reflect higher levels of supplemental and by-pass feeding. From a biosynthesis perspective high levels of preformed fat entering the mammary cells will have a tendency to inhibit the enzyme acetyl CoA-carboxylase that is a critical first step in *DeNovo* synthesis of FA from acetate in the mammary tissue.

Milk Protein

At the beginning of the survey we had not focused on bulk tank milk protein content, but we were collecting the data. Upon analysis of the data we were surprised by the high positive correlation between milk FA composition and bulk tank true protein concentration. The relationship between the g/100 g of *DeNovo* FA in milk and bulk tank milk protein content is shown in Figure 6. There is a positive correlation of increasing bulk tank true protein test with increasing *DeNovo* FA concentration in milk in both Holstein and Jersey milks. This was not expected. The range from high to low in bulk tank true protein concentration was large within both the Jersey and Holstein breeds and would have a significant impact on cheese production and composition of dried milk protein ingredient products.

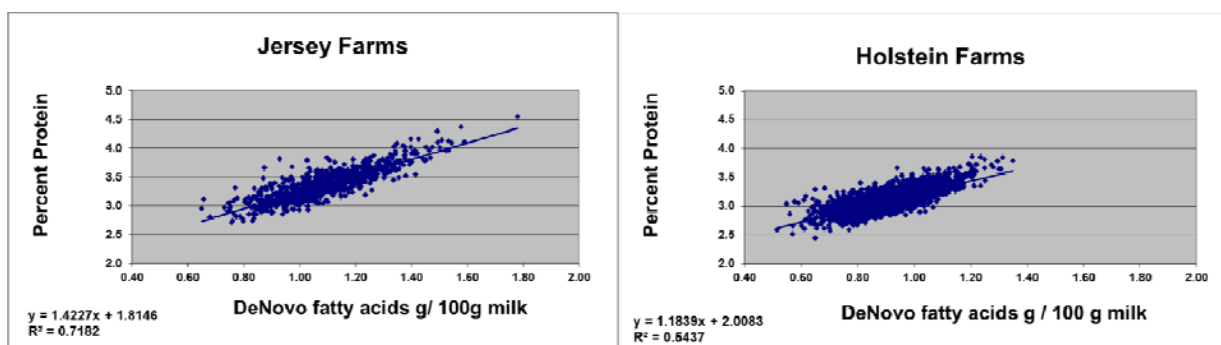


Figure 6. Bulk tank true protein test (%) for Jersey and Holstein herds plotted as a function of milk *DeNovo* fatty acid content.

Why did we observe a strong positive correlation between *DeNovo* FA concentration in milk and milk protein concentration? Protein is synthesized with the mammary tissue from amino acids. Preformed milk proteins do not enter the mammary tissue from the blood. There are 2 groups of amino acids used to synthesize milk proteins: essential amino acids and nonessential amino acids. The nonessential amino acids can be produced throughout the cow's body and in the mammary tissue. The essential amino acids have to come from the diet. Generally, free amino acids in the rumen are rapidly metabolized by the rumen microflora. There are 3 sources of rumen undegradable (or by-pass) protein: proteins in the diet that do not degrade in the rumen at neutral pH, proteins containing essential amino acids that are part of the cellular biomass produced during rumen fermentation, and protected amino acids fed in the diet. When rumen function and fermentation of digestible carbohydrates is working well then the rumen microflora biomass (i.e., essential amino acids) and rumen volatile FA (i.e., butyrate, acetate, and propionate) should be maximized. Therefore, *DeNovo* FA output in g/100 g milk may be an indicator of both excellent production of volatile FA and microbial biomass providing a rich source of essential amino acids in support of milk protein synthesis.

SUMMARY

The key FA parameter that was positively correlated with bulk tank milk fat and true protein concentration was *DeNovo* FA (g/100 g milk). Structural parameters of FA chain length (carbon number) and total unsaturation (double bonds /FA) were negatively correlated with fat and protein (g/100 g milk). This was true for both Jersey and Holstein. When *DeNovo* FA (relative % of FA) were higher, fat test was higher for both Jersey and Holstein. As *DeNovo* FA (g/100 g milk) increased, fat (g/100 g milk) increased ($P < 0.001$) at a much faster rate (i.e., higher slope) than when preformed FA (g/100 g milk) increased (slope 2.28 vs. 1.29) for Jersey and for Holstein (slope 2.16 vs. 1.22), for *DeNovo* vs. preformed, respectively. As the proportion of *DeNovo* FA increased (and fat percent increased), the measured FA chain length and double bonds per FA decreased ($P < 0.001$). True protein (g/100 g milk) increased as *DeNovo* FA (g/100 g milk) increased. What we do not know from this work is if the production of milk components per cow per day are higher when *DeNovo* FA as a proportion of total FA is higher. This will be critical in determining if feeding and management strategies to increase *DeNovo* FA production per day will also increase output of fat and true protein per cow per day. That will be the focus of a follow-up farm management field study.

FUTURE WORK

Farm Management Study

We hypothesize that feeding and farm management practices influenced *DeNovo* FA production and milk fat and protein (g/100 g milk) by influencing the volatile FA production in the rumen and microbial biomass. A group of 20 Jersey and 20 Holstein farms of interest that had a wide range of *DeNovo* FA (g/100 g fat) and grams of *DeNovo* FA per 100 g of milk fat were selected for a more in-depth field study, that began in April 2014, to determine if there are cost effective feeding and management practices that can be used to increase fat and protein tests based on monitoring milk FA composition. During the 14 month period of our study, the 10 Holstein and 10 Jersey low *DeNovo* herds averaged 3.62 and 3.97% fat and 2.99 and 3.15% true protein, while the 10 high *DeNovo* Holstein and Jersey herds averaged 3.92 and 4.80% fat and 3.09 and 3.62% true protein, respectively, as shown below in Table 1. A field study is being conducted to identify feeding and management practices that produce differences in milk FA composition and milk component concentrations at the bulk tank level.

Table 1. Mean milk composition for 10 low and high *DeNovo* Holstein and 10 high and low *DeNovo* Jersey farms and the mean relative proportion of the total FA in the milk that are *DeNovo*, mixed origin and preformed for the period July 2012 through August 2013.

	Group		Anydrous	True		Relative Percent of total FA		
Breed	DeNovo	Fat	Lactose	Protein		Denovo	Mixed	Preformed
Holstein	Low DN	3.62	4.60	2.99		24.08	34.00	41.92
Holstein	High DN	3.97	4.56	3.15		26.08	35.08	38.84
Jersey	Low DN	3.92	4.53	3.09		25.04	33.35	41.61
Jersey	High DN	4.80	4.54	3.62		27.41	34.62	37.96

Milk Testing for Individual Cows

Going forward, this work is leading to individual cow milk testing directly on large farms within the US to provide real-time farm management data. Concepts for integration of mid-IR milk analysis directly into the milking systems on large farms are being considered. The combination of milk weight and the component concentrations (i.e., fat, protein, lactose, and milk NPN/Urea content) will allow calculation of energy output in the milk and in combination with feed input data will allow an estimate of energy and protein balance of individuals or groups of cows within the herd.

Some other measures that we have developed for use in individual cow milk testing that are blood BHB and blood nonesterified fatty acids (NEFA) for ketosis prediction, in addition to milk BHB and acetone concentrations. The measurement and rate of change of blood NEFA estimated from every milking analysis of milk will provide a view of the metabolic status combined with energy balance estimates will provide indices of potential first insemination success rate in breeding and the potential to identify individual cows where a delay in breeding might be the most economically correct management decision. Indirect measurement of rumen pH through milk analysis might provide insight into how a cow is interacting the complex mixture of nutrients in the rumen, as that impacts the chemistry of the milk.

Measurements of milk *trans* FA that predict classical milk fat depression using a ratio of C18:1 *trans* 10 to C18:1 *trans* 11 isomers could be very useful in identification of those cows that are predisposed to milk fat depression on certain types of feeding strategies. It may be possible to develop a milk analysis model to provide an index of rumen pH that could be useful when making changes in rations. It could be that relationships with animal genetics will become apparent when large data sets are available. Combinations of individual parameters that provide more predictive indices of feed efficiency, ketosis, and probability of successful breeding may be derived from the current PLS models for milk analysis. In the future, development of models to determine pregnancy status and loss of pregnancy will bring further benefit in the applications of mid-IR milk testing for real-time farm management milk testing.

ACKNOWLEDGMENTS

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